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CUMULATIVE TESTING EXPERIENCE WITH CONSECUTIVE LOTS OF ORAL POLIOMYELITIS VACCINE

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Any vaccine proposed for general and continued use must first satisfy certain laboratory criteria of potency and safety of successive lots produced over a period of time. Oral poliomyelitis vaccine is no exception to this rule, though specific production and testing standards for it have not yet been established.

A number of safeguards are obvious prerequisites to the release of a given production lot for clinical trials. For the most part, these were suggested by experience with other vaccines also prepared in monkey-kidney culture. They are particularly critical in the case of oral poliomyelitis vaccine, which cannot contain a preservative that would control undesirable accompanying micro-organisms. The measures taken must be so applied as to rule out, with a well-founded degree of certainty, the presence in the vaccine of any organism, harmful or not, other than poliovirus. They also must serve to ascertain that the avirulence of the vaccine virus remains unchanged during the course of repeated cultivation, and, under ideal conditions, they should permit measurement of the immunogenic potency of the vaccine batch under test.

With oral poliomyelitis vaccine only the first of these three requirements can be carried out in the laboratory under conditions reflecting reality. The other two questions, of vaccine safety and potency for man via the oral route, could perhaps be answered only by use of the chimpanzee in prohibitive and unrealistic numbers. Consequently, for the answer regarding avirulence, rather artificial and sometimes drastic methods of inoculation of certain species of monkeys, such as the rhesus or the cynomolgus, must be resorted to. And since in the case of oral poliomyelitis vaccine antigenic stimulation is a function of *in vivo* viral multiplication, determination of the virus content of the vaccine is a poor but, in the circumstances, necessary and logical substitute.

Though our laboratories have been engaged in the study of a live virus oral poliomyelitis vaccine since the late 1940s, this report will attempt to deal only with our experience since 1957 in the production and testing of serial lots of vaccine, some of which were used in small- and large-scale clinical trials. This experience will be correlated to the immune status of, and the

clinical results obtained in, the populations fed, in an effort to determine to just what degree monkey tests can measure the safety of the vaccine virus for man.

Virus Strains

The origin and development of the Lederle strains of attenuated polioviruses have been amply described and summarized in previous reports from this laboratory and elsewhere (Martins da Silva *et al.*, 1957; Koprowski, 1958; Abad-Gomez *et al.*, 1959; Barr *et al.*, 1959). A brief up-to-date résumé follows.

Type I virus originated from a mixture of tissue-culture fluids respectively infected with the Sickle and Mahoney type I isolates (Chart 1) (Koprowski *et al.*, 1954). The strain, derived from 26 serial intraspinal passages in mice, was subsequently transferred to monkey-kidney-tissue culture for 10 generations, was passed 14 times in chick-embryo-tissue culture, and was plaqued out on monkey-kidney monolayers. After five alternations between monkey-kidney and chick-embryo cultures, four additional plaquings were carried out before selection of the original seed virus designated Pool 400B-10-M3. A fifth plaquing, derived from that pool, was the source of a second seed pool (7-1231-114). Intracerebral and intraspinal inoculations of monkeys with plaque-derived virus pools repeatedly demonstrated at all passage levels the nearly complete loss of virulence of the virus when inoculated intracerebrally, and a considerable diminution of its intraspinal activity.

Type II virus, as indicated in Chart 2, descended from the MEF1 strain following its intracerebral passage 157 times in suckling hamsters, its adaptation to and propagation in chick embryos for 17 consecutive generations, and its passage in monkey-kidney cultures (Cabasso *et al.*, 1952; Moyer *et al.*, 1952; Roca-Garcia *et al.*, 1952). Seed virus pool 7-1232-200 was obtained after 11 transfers and two consecutive plaquings in this latter host cell. The reason for growing the virus back in monkey-kidney-tissue culture instead of the chick embryo as the source of vaccine was the relatively low titres achieved in chick embryos, which rarely approached $10^{6.0}$ mouse LD_{50} per ml. of a 20% suspension.

The original type III strain was an essentially avirulent variant isolated in monkey-kidney-tissue culture by Dr. John P. Fox

from the stool of a healthy carrier (Chart 3). A monkey-kidney culture pool infected with the progeny of the fourth plaquing of this strain constituted the original seed virus, pool 7-1233-310. A second seed pool (7-1233-310E5) was obtained with one additional plaquing from the first pool.

Monkey virulence tests on both type II and type III viruses between the various plaquings yielded results essentially similar to those with the type I strain.

Production of Vaccine

This report concerns the production and testing of a total of 31 seed and vaccine lots of the three types of virus.

The genealogy and distribution of type I vaccines in various clinical trials are illustrated in Chart 4. It can be seen that seven vaccine batches originated from two seed lots, and the second seed virus was only one plaquing removed from the first. Four of these vaccines were fed in eight separate trials carried out in seven different countries. In all but one of the trials, single lots of vaccine were used throughout each trial. However, in Uruguay, because of the large number of individuals vaccinated, vaccines from four different lots were employed.

CHART 1.—Passage history of Lederle attenuated poliovirus (SM strain, type I).

CHART 2.—Passage history of Lederle attenuated poliovirus (MEF1 strain, type II).

The history of type II vaccine production and clinical trials is shown in Chart 5. Here a single seed virus was the progenitor of all 10 vaccine lots produced, and four of these lots were used in the field studies.

For type III, as indicated in Chart 6, production and trial histories of the vaccine are similar to those of type I in that two closely related seed pools were used for the 10 lots produced. Two of the lots were large enough and of sufficient titres to satisfy the needs of all clinical trials undertaken thus far.

All of the vaccine lots were produced in monkey-kidney tissue. Povitsky bottle and tube cultures were prepared according to the method of Dulbecco and Vogt (1954), using multiple trypsinizations at room temperature or the overnight technique at 4° C. suggested by Bodian (1956). Growth fluid consisted of Earle's basal medium to which were added Eagle's vitamin mixture, 0.5% lactalbumin hydrolysate, and 2% calf serum (Earle, 1943; Eagle, 1955). The sodium bicarbonate content of the fluid was 0.11%. The

growth medium was dispensed at the rate of 100 ml. per Povitsky bottle of 2,000-ml. capacity, or 1 ml. per tube. The various lots of calf serum employed were routinely tested for poliovirus "neutralizing substances" before their addition to the growth medium, and those with titres higher than 1:8 were discarded.

Monolayers were allowed to grow at 37° C. for six days before introduction of the virus. Since experimental evidence had shown that cultures which were "leached out" with renewal fluid before inoculation did not yield substantially higher titres than those where leaching was omitted, inoculation with virus was routinely carried out after simple removal of the growth medium and its replacement with renewal solution in amounts of 500 ml. per Povitsky bottle and 1 ml. per tube.

The renewal fluid also was made up of Earle's basal solution except that the sodium bicarbonate concentration of 0.22% was retained and the serum was omitted. In addition, it contained the following substances: 0.1%

yeast extract, 0.1% bovine albumin, 0.03% L-glutamine, and 0.5% lactalbumin hydrolysate. Its pH was between 7.4 and 7.6. Antibiotics were added in the following concentrations per ml. of either growth or renewal medium: 50 units of penicillin, 50 µg. of streptomycin, 25 µg. of neomycin, and 50 units of mycostatin.

As an additional safety precaution, each pair of monkey kidneys was processed separately, and the bottles derived from it provided individual vaccine harvests. In most instances not less than 1 ml. of undiluted seed virus was used for inoculation of the culture bottles. These were incubated at 37° C. and examined daily for progress of viral effect. Bottles showing the slightest evidence of bacterial or fungal contamination were immediately discarded. The others were harvested on the third or fourth day, when the monolayer had completely detached from the glass. As already mentioned, all cultures from one monkey were harvested and pooled separately, and were given an identifying pool number. The pH of each pool was determined, and if necessary it was adjusted back to 7 with M/2 monobasic sodium

Monkey Virulence Test				Human Stool (Fox P1149)	Monkey-Kidney- Tissue Culture	Times Plaques in Monkey- Kidney Cells
Logs of Virus Inoculated	Intra- Cerebral Paralytic Ratio	Logs of Virus Inoculated	Intra- Spinal Paralytic Ratio			
6.9	0/7	6.2	2/4	↓	3 pas.	→ No. 1
7.7	1/14	7.0	1/8	↓	1 pas.	
5.7	0/10	6.0	1/4	↓	1 pas. (7.2)*	→ No. 2
5.7	0/4	5.0	0/4	↓	2 pas.	
4.7	0/4	4.0	0/3	↓	1 pas. (8.0)*	→ No. 3
				↓	1 pas.	
6.4	0.5	5.7	0/5	↓	1 pas. (6.7)*	
7.2	0/14	6.5	0/4	↓	1 pas.	
6.2	0/4	5.5	0/4	↓	1 pas. (7.5)*	→ No. 4
5.2	1/4	4.5	0/4	↓	2 pas.	
4.2	0/4	-	-	↓	1 pas.	
7.5	0/5	6.5	0/5	↓	1 pas. (7.5)*	→ No. 5
6.5	0/4	5.5	27/5	↓	Pool 7-1233-310 (Seed virus)	
		4.5	3/5	↓		
7.2	17/10	6.2	2 17/10	↓	2 pas.	
6.2	0/4	5.2	0/5	↓	Pool 7-1233-310 (7.2)*	
5.2	0/6	4.2	0/5	↓	(Seed virus)	

*Log. TCD₅₀ per ml.

CHART 3.—Passage history of Lederle attenuated poliovirus (Fox strain, type III).

CHART 4.—Production summary of type I of oral poliomyelitis vaccine.

phosphate solution. The pool was then filtered through a Hormon D9 asbestos pad and held overnight at 4° C. On the following day the pH was rechecked, and readjusted if necessary. Samples for the various *in vitro* tests were removed, and the bulk of the pool was stored in a -20° C. freezer.

To ensure ready identification and to minimize errors, especially when working on a fairly large scale, not only were samples and bulk harvests marked with the virus type and pool number, but this information was written on large labels of different bright colours assigned from the start to the three types of virus: red for type I, yellow for type II, and green for type III. These identifying colours followed the respective virus types to the final containers in which they were shipped.

Testing of the vaccine was carried out in two steps. First, the undiluted individual monkey harvests were subjected to a battery of *in vitro* tests for the purpose of ruling out the presence of bacterial or fungal contaminants and of simian and measles viruses. In addition, viral titration and identification tests were performed. When the results of all these tests were considered satisfactory, testing was continued by *in vivo* methods, in some cases on the individual monkey harvests, but more frequently on 16- to 20-litre vaccine lots made by pooling two to four individual harvests.

The second series of tests was designed to rule out the presence of C.N.S.-herpes, Coxsackie, and B viruses, as well as the tubercle bacillus. Finally the neurovirulence test was performed in cynomolgus monkeys.

Upon completion of all tests, satisfactory vaccine lots were diluted to the desired virus concentration in one of two ways: either by absorption on granulated gelatin to obtain a relatively dry, free-flowing preparation which could be filled into hard-shell gelatin capsules, or by mixing the fluid with a flavoured and stabilizing syrupy excipient.

Testing of the Vaccine

The *in vitro* testing schedule for individual monkey harvests is summarized in Table I. In brief, each preparation is tested for bacterial sterility in thioglycolate broth, at both room temperature (22-24° C.) and 37° C. A similar test for fungi is performed in Sabouraud's broth.

Testing for simian viruses is carried out with specially prepared antisera. When using monkey-kidney-propagated poliovirus for the hyperimmunization of laboratory animals, it is difficult to exclude the possibility of a concomitant simian viral agent, and antibodies against it may be elicited simultaneously with those produced against poliovirus. Similarly, monkeys

are unsuitable for preparation of the antisera to be used in this test, since they may already have simian viral antibodies from naturally acquired infections. Consequently we have adopted the following routine procedure. The three types of poliovirus are "purified" by at least three, and in some cases up to eight, terminal dilution passages in HeLa cultures. These latter are not known to be susceptible to most simian viruses, and therefore should yield poliovirus pools reasonably free from other contaminating viruses. Such pools were used in adjuvant mixtures for the hyperimmunization of donor rabbits or chickens.

The simian virus exclusion test is performed by mixing the undiluted vaccine sample with an equal volume of undiluted homologous antiserum. After three hours

CHART 5.—Production summary of type II oral poliomyelitis vaccine.

incubation at room temperature, the virus-serum mixture is inoculated into 10 monkey-kidney-culture tubes in amounts of 0.2 ml. per tube. These are incubated at 37° C. and examined daily for cytopathology. At the end of seven days the fluid is removed from these tubes, pooled, and reinoculated into 10 additional freshly prepared monkey-kidney-culture tubes, while the original set of tubes is renewed with

TABLE I.—*In vitro* Tests on Individual Monkey Harvests (3- to 10-litre Lots)

Purpose of Test	Test System	Observation (Days)
To rule out:		
1. Bacterial contaminants	Thioglycolate broth—room temperature (22-24° C.) and 37° C.	14
2. Fungal contaminants	Sabouraud's broth—room temperature (22-24° C.) and 37° C.	14
3. Simian viruses	(a) Vaccine-homologous antiserum mixture in MK tubes	14
	(b) Passage of fluid from a in second set of MK tubes on 7th day	7
4. Measles virus	Vaccine-homologous antiserum mixture in HeLa cultures	14
To determine:		
1. Poliovirus titre	Tenfold dilutions in MK tubes—12 tubes per dilution	8
2. Poliovirus identity	Mixtures of vaccine and antisera to 3 types of virus in MK cultures	8

fresh medium. Five to 10% homologous antiserum is added to the 20 tubes, which then are returned to the incubator at 37° C. and observed for another seven-day period. When, occasionally, an apparent virus breakthrough occurs, the resulting agent is grown out and resubmitted to the same procedure. It should be added that throughout this set of tests proper controls are always included.

For the exclusion of measles virus, the initial virus-homologous-antiserum mixture referred to above is also inoculated into 10 HeLa-culture tubes. These are incubated at 37° C. for 14 days and observed for the appearance of any multinucleated giant cells.

Both titration and identification of the virus are made in tubes of monkey-kidney cultures after eight days of

Vaccine lots which pass all of the *in vitro* tests are then subjected to the tests indicated in Table II.

Absence of C.N.S. and herpes viruses is determined by inoculating each of 12 adult mice with 0.03 ml. of undiluted vaccine intracerebrally and 0.1 ml. intraperitoneally. The mice should show no evidence of disturbance of the central nervous system during a period of 21 days. To rule out the presence of Coxsackie viruses, at least three litters of suckling mice are used. Each animal receives 0.01 ml. of undiluted vaccine intracerebrally and 0.1 ml. intraperitoneally, and should remain normal for 21 days. It should be noted that, as the MEF1 type II strain used in this laboratory still possesses its mouse marker, the C.N.S.-herpes and Coxsackie exclusion tests have to be carried out in the presence of homologous antiserum.

For the detection of B virus, three 4- to 6-lb. (1,815 to 2,720-g.) rabbits are each given 0.25 ml. of the bulk vaccine intracerebrally, 9 ml. subcutaneously, and four intradermal injections of 0.25 ml. each, administered at four different sites. These animals are also observed for 21 days, during which time they should be free of paralysis and of necrosis at the sites of inoculation.

One test for the tubercle bacillus consists of inoculating three 300- to 500-g. guinea-pigs with 0.2 ml. of undiluted vaccine intracerebrally and 1 ml. intraperitoneally. The animals should show no clinical evidence of disease during a 42-day observation period. They are then sacrificed and

examined for gross pathological signs of tuberculosis, and smears are made from the meninges under the medulla, to be examined for acid-fast organisms. In addition to the *in vivo* test, tubes of Lowenstein-Jensen medium are each inoculated with 0.5 ml. of vaccine and incubated for 42 days at 37° C.

The series of *in vivo* tests is completed with the checking of the vaccine for neurovirulence in cynomolgus monkeys. The animals that have been used weighed between 2.5 and 5 lb. (1,135 and 2,270 g.) and were received direct from the Philippine Islands. During the past year their average size was somewhat smaller than is desirable, and some groups reached the laboratory in rather poor condition. Despite intensive conditioning care soon after their arrival, an appreciable number were lost before inoculation, during the first two to three weeks; in some groups this number amounted to 20% of the total. All monkeys were tuberculin-tested on arrival and were not used for testing for at least three weeks thereafter. The neurovirulence test was carried out by both the intracerebral and the intraspinal route.

CHART 6.—Production summary of type III oral poliomyelitis vaccine.

incubation at 37° C. For titration, ten-fold dilutions are used, with 0.1-ml. inocula and 12 tubes per dilution. Titres are calculated by the Reed and Muench (1938) formula. Identification, the final *in vitro* test, employs mixtures of vaccine and antisera to the three types of virus.

TABLE II.—*In vivo* and Other Tests on Pools of 2 to 4 Individual Monkey Harvests (16- to 20-litre Lots)

Purpose of Test	Test System	Observation (Days)
To rule out:	1. C.N.S.-herpes viruses	21
	2. Coxsackie viruses	
	3. B. virus	21
	4. Tubercle bacillus	42
To determine:	(a) Guinea-pigs (3)—I.C. and I.P.	42
	(b) Lowenstein-Jensen medium	42
	(a) I.C., undil.—10, 10 ⁻¹ -6, 10 ⁻² -6 (0.5 ml. bilaterally)	28
	(b) I.S., undil.—10, 10 ⁻¹ -6, 10 ⁻² -6 (0.1 ml.)	21
Monkey neurovirulence*	(c) Histopathological examination of cervical and lumbar enlargements of all monkeys	

* All tests performed in cynomolgus monkeys.

Intracerebral Monkey Inoculation

Of the vaccine pools reported on here, all type I lots (8 in number), 7 of the 11 type II lots, and 3 of the 11 type III lots were inoculated into the thalamic region in 0.5-ml. amounts on each side. Four type II and four type III lots were inoculated in 0.5-ml. amounts in only one site, and the remaining four type III pools were also injected in one site, but in amounts of 1 ml. The extent of traumatic loss of animals varied but little when using these various modes of inoculation, and rarely exceeded an occasional monkey out of groups of 22 to 30.

At different times during the course of the observation period a number of test monkeys were found dead, most of them without having shown any paralysis and a few with a slight weakness or paralysis of one limb. In most instances necropsy revealed pneumonia or extremely heavy worm infestation or both. In any event, the spinal cords of all these animals were sectioned for histopathological studies, as were those of all survivors on the 28th day of observation.

That most of these early deaths were in no way related to poliomyelitis is borne out by the histopathological results summarized in Tables III, IV, and V.

Table III indicates that of 211 monkeys inoculated intracerebrally with type I vaccines, 17 (8%) died before the end of the observation period. Only one death,

TABLE III.—*Intracerebral Test with Type I Vaccine Lots: Monkeys Dead Before End of Observation Period*

Lot No.	No. of Monkeys Tested	Dead Before 28 Days		Ratio		
		No. of Monkeys	Time of Death (in Days)	Para-lytic	Polio Histopathological*	
					Cervical	Lumbar
110	25	1	1-5	0/1	0/1	0/1
		1	6-10	0/1	0/1	0/1
114	30	2	11-20	0/2	0/2	0/2
115	30	1	11-20	0/1	0/1	0/1
120	30	0	—	—	—	—
121	30	0	—	—	—	—
126	22	1	6-10	0/1	0/1	0/1
		1	11-20	0/1	0/1	0/1
		1	21-28	0/1	0/1	0/1
140	22	0	—	—	—	—
162P†	22	4	6-10	0/4	0/4	1/4
		5	11-20	1/5	1+1/5	2+1/5
Totals	211	17	—	17/17	1+1/17	3+1/17

Percentage dead: 8.0.

* All histopathological reactions were of the inflammatory type; no neuronal loss or damage was observed.

† Scored histopathologically by method B. All others scored by method A.

which might be considered traumatic, occurred within five days of inoculation, all the others occurring after the sixth and up to the 28th day. Only one of the 17 monkeys had shown any, and that a questionable, sign of paralysis of one limb before death, and 13 were histologically negative for poliomyelitis. Of the four positive monkeys, none had any neuronal destruction, and all reactions observed were of the inflammatory type.

Table IV shows that of 314 monkeys inoculated intracerebrally with type II vaccine, 39 (12.4%) died before the end of the observation period. Only seven of these deaths occurred during the first five days and can be considered traumatic. Of the 32 animals that died after the sixth day, two were suspected of some degree of paralysis of one limb before death, and two of only weakness of one limb. On histological examination 11 animals showed some neuronal loss or inflammation; the others were completely negative.

TABLE IV.—*Intracerebral Test with Type II Vaccine Lots: Monkeys Dead Before End of Observation Period*

Lot No.	No. of Monkeys Tested	Dead Before 28 Days		Ratio		
		No. of Monkeys	Time of Death (in Days)	Para-lytic	Polio Histopathological*	
					Cervical	Lumbar
200	30	1	6-10	0/1	0/1	0/1
		1	11-20	1/1	0/1	0/1
213	30	1	1-5	0/1	1/1	1/1
		3	11-20	0/3	2/2	2/2
216	60	4	6-10	1/4	1/3	2/3
		1	11-20	1/1	0/1	0/1
217A	40	1	1-5	0/1	0/1	0/1
		1	6-10	0/1	0/1	0/1
		3	11-20	0/3	1/3	1/3
222	22	1	1-5	0/1	0/1	0/1
227	22	1	6-10	0/1	1/1	1/1
228	22	0	—	—	—	—
240†	22	1	1-5	0/1	0/1	0/1
		3	11-20	0/3	0/3	0/3
		1	21-28	0/1	0/1	0/1
241†	22	2	1-5	0/2	0/2	0/2
		2	6-10	0/2	0/2	0/2
		2	11-20	1/2	1/2	1/2
		2	21-28	0/2	0/2	0/2
242†	22	1	1-5	0/1	0/1	0/1
		1	6-10	0/1	0/1	1/1
		2	11-20	0/2	1/2	1/2
243†	22	1	21-28	0/1	0/1	1/1
		3	11-20	0/3	0/3	0/3
Totals	314	39	—	2+27/39	7+17/37	8+37/37

Percentage dead: 12.4.

* All histopathological reactions were of the inflammatory type; no neuronal loss or damage was observed.

† Scored histopathologically by method B. All others scored by method A.

However, as indicated later, neuronal damage in the positive animals was of the same degree as that found in surviving non-paralysed animals inoculated with some lots of type II vaccine.

Table V summarizes the early deaths of monkeys inoculated with type III vaccine. Here, 17 of 246 animals (6.9%) died before the 28th day, three of them within the first five days. In one of these three, weakness of one limb was observed on the day following inoculation and, as suggested by a completely negative histopathology of the cord, must have been due to trauma. The histopathological reactions observed in three of the animals were of the inflammatory type, and no neuronal loss or damage was noted.

All vaccine lots were inoculated intracerebrally both in undiluted form and as 10^{-1} and 10^{-2} dilutions. Early batches were administered to 10 monkeys per dilution,

TABLE V.—*Intracerebral Test with Type III Vaccine Lots: Monkeys Dead Before End of Observation Period*

Lot No.	No. of Monkeys Tested	Dead Before 28 Days		Ratio		
		No. of Monkeys	Time of Death (in Days)	Para-lytic	Polio Histopathological*	
					Cervical	Lumbar
310†	10	1	11-20	0/1	0/1	0/1
318†	30	2	1-5	0/2	0/2	0/2
		2	11-20	0/2	0/2	0/2
319†	30	0	—	—	—	—
334	22	0	—	—	—	—
341	22	0	—	—	—	—
342	22	2	11-20	0/2	0/2	0/2
344	22	1	11-20	0/1	0/1	0/1
352P	22	1	1-5	1/1	1/1	0/1
		2	1-5	1/2	0/2	0/2
360P	22	1	6-10	0/1	0/1	0/1
		1	11-20	0/1	1/1	1/1
361P	22	1	6-10	0/1	0/1	0/1
362P	22	1	6-10	0/1	0/1	0/1
		2	21-28	0/2	1/2	0/2
Totals	246	17	—	27/17	2+17/17	1/17

Percentage dead: 6.9.

* All histopathological reactions were of the inflammatory type; no neuronal loss or damage was observed.

† Scored histopathologically by method A. All others scored by method B.

and some have had repeat tests. The more recent routine has consisted of inoculating the undiluted vaccine into 10 monkeys, and the 10^{-1} and 10^{-2} dilutions into groups of six animals each. Inoculated monkeys were closely examined daily for 28 days for signs of limb weakness or paralysis, by exercising them in their runs. Any suggestive clinical signs were carefully recorded.

Intraspinal Monkey Inoculation

Most vaccine lots included in this report were inoculated intraspinally by means of a 26-gauge needle. These tests were carried out before the recent studies which revealed the influence of the needle gauge on the outcome of the test (Melnick, 1959; Sabin, 1959). Since we obtained this information the new vaccine lots tested have been inoculated with a 20-gauge needle, though in some instances 20- and 27-gauge needle techniques were compared.

Regardless of the gauge of the needle, as in the intracerebral test with early batches of vaccine, 0.1 ml. of undiluted tissue-culture fluid and of 10^{-1} and 10^{-2} dilutions was injected into the lumbar enlargement of groups of 10 animals per virus dilution. More recent lots of vaccine have been injected into 10, 6, and 6 monkeys per undiluted and 10^{-1} and 10^{-2} dilutions, respectively. *

A question has lately been raised of whether, in the early days of our work with this technique, the fourth intervertebral space above the iliac crest was always the site of the inoculation. Review of histopathological specimens indicated that the correct site apparently was hit in at least 71% of the cases and that in about 5% the needle track was in the white instead of the grey matter; no needle track could be seen in the rest (24%). Evidence also was obtained that in a few cases inoculation was into the fifth intervertebral space. However, of late both the iliac crest and the level of the last ribs have been used as landmarks for location of the inoculation site, and the record of properly located inoculations has been greatly improved. Other details pertaining to this procedure of intraspinal inoculation of monkeys have been described in detail by Sabin (1959). It should be added that, regardless of the care with which this technique is applied, the rate of traumatic paralysis is always greater than that following intracerebral inoculation.

Twenty-four hours after inoculation, and daily thereafter, inoculated monkeys are exercised to observe any deviation from normal gait and comportment. Signs of weakness or paralysis on the very first day are considered to have been caused by trauma, unless on subsequent days evident progress of the paralysis is observed. Cords of animals dead of undetermined causes during the observation period are saved for histopathology, regardless of whether paralysis was present prior to death. Animals showing advanced degrees of weakness or paralysis before termination of the test are sacrificed, examined *post mortem*, and their cords are removed for histopathology. The test is terminated on the 21st day, when all survivors are sacrificed and subjected to gross pathological examinations; all cords are placed in 10% formalin and processed for microscopical histopathological examination.

Neuro-Histopathology

Two methods were used for histological study. With Method A, fixed cervical and lumbar enlargements

were cut into 1-cm. blocks. These were embedded in celloidin and cut serially into sections 12 microns thick. Every 20th section was stained by Nissl's method and examined. Scoring was essentially based on the estimated percentage of nerve cells lost from the anterior horns, and was as follows: 0=no cell loss and no inflammatory reaction; $\pm$ =no significant cell loss but some inflammatory reactions such as perivascular infiltration with or without glial nodules; $+$ =10-20% cell loss*; $++$ =21-40% cell loss; $+++$ =41-60% cell loss; $++++$ =61-80% cell loss; $+++++$ =above 80% cell loss.

Method B, which has been adopted in our laboratory only recently, follows the directions described by Sabin (1959) and Melnick (1959). Scoring by this method is as follows: 0=no lesions; $+$ =single focal neuronal lesion, single vascular lesion, or single area of cellular infiltration; $++$ =combination of two or three of the above lesions; $+++$ =cluster of neuronal lesions plus more extensive vascular lesions and areas of cellular infiltration; $++++$ =gross destruction of nerve cells plus extensive areas of infiltration and perivascular cuffing. In addition, this method recommends that if no needle track can be discerned in the anterior horn of the lumbar cord of a monkey injected intraspinally, this animal be not considered in the final tally of results.

Test Results

Freedom from contaminating bacteria, moulds, and viruses was general for all tested vaccine lots. In very few cases a *Pseudomonas* organism of the *fluorescens* group was recovered from individual monkey harvests, but this was readily cleared up by an additional filtration before submission of the lot to other tests. In no instance were tubercle bacilli or C.N.S., herpes, Coxsackie, or B viruses encountered. Similarly, except for the so-called "foamy agent," which also was seen quite consistently in control cultures, no recognizable simian viruses were isolated, despite the very large number of tests.

Effectiveness of the simian-virus-exclusion test system employed was checked in two ways. First, samples of a type III vaccine pool were deliberately contaminated with decreasing amounts of SV_{15} .† The mixtures were then submitted to the routine exclusion test, in the presence of type III antiserum, alongside an uncontaminated sample of the same type III pool. Within the 14-day observation period SV_{15} had been recovered from every mixture expected to contain it, while the uncontaminated sample gave no evidence of virus growth. In a second set of experiments the hyperimmune sera used in the routine test were assayed for neutralizing action on at least six known isolates of simian viruses: SV_2 , SV_4 , SV_6 , SV_{15} , SV_{26} , and SV_{28} . In every instance simian virus titres were of the same order, whether in the presence of normal or of poliomyelitis hyperimmune chicken sera. This study is being extended to other known simian virus strains with both chicken and rabbit poliomyelitis antisera.

Details pertaining to the date of preparation, volume, and titres of type I vaccine pools are given in Table VI. The period covered is two years, during which time over 113 litres of vaccine were produced and completely

*Cell loss below 10% was considered to be within experimental error.

†We are indebted to Drs. Samuel Bozeman, of Pitman-Moore Company, and Robert Hull, of Eli Lilly Company, for the various isolates of simian viruses received from them.

tested. TCD₅₀ titres ranged from 10^{7.3} to 10^{8.2} per ml. At the rate of 10^{6.0} TCD₅₀ units per vaccination the total volume produced should be sufficient for 4½ million doses.

Similar details are summarized in Table VII for type II vaccines. The 11 lots amounted to 120 litres, ranging in titre from 10^{6.8} to 10^{7.6} TCD₅₀ per ml. Because of the somewhat lower average titre, this quantity of vaccine, though slightly greater than for type I, would yield approximately 2,600,000 clinical doses.

A summary of type III vaccine lots is shown in Table VIII. The total volume produced is 143 litres of

an average titre of 10^{7.5} TCD₅₀ per ml., also amounting to about 4½ million immunizations.

Results of the intracerebral inoculation of monkeys with the eight lots of type I vaccine are shown in Table IX. It can be seen that only 3 of the 195 inoculated monkeys still alive on the 28th day of observation showed either a frank paralysis or a questionable involvement of a single limb. These reactions occurred between the fourth and sixth days following inoculation and progressed no further. Moreover, the three animals were negative for histopathological changes in their cervical and lumbar enlargements. In fact, out of the 195 monkeys, neuronal damage was observed only in scattered animals, none of which showed paralysis. The damage ranged from a single focus of inflammation without cellular loss ($\pm$ or questionable on the table) to not more than 2+ (equivalent to 20 to 40% cellular loss). In all, 10 animals (4.8%) showed such changes in the cervical and 15 (7.2%) in the lumbar enlargement.

Table X summarizes results of the intraspinal monkey test with the same lots of type I vaccine. The number of animals showing paralysis is undoubtedly far greater than with intracerebral inoculation. With undiluted vaccines 37/104 animals were affected (35.5%); with 10⁻¹ dilutions, 35/73 (47.9%); and with 10⁻² dilutions, 24/83 (28.8%). Most cases were limited to the lower extremities, and ranged from weakness of a single limb to complete paralysis of both legs. Death of an affected animal was exceptional. The great majority (260/272) survived the 21-day observation period and were sacrificed for histopathology. Understandably, monkeys inoculated intraspinally showed marked histopathological changes. Cellular loss and inflammatory reactions ranged from absent or minimal to total. The rate of change was greater in the lumbar enlargement, the site of inoculation, than in the cervical, averaging for the former 65.5% of the animals and for the latter 42%. Similarly, the change tended to be greater in one anterior horn than the other, particularly in the cervical region. It may also be noted that while 65.5% of the animals had some degree of histopathological change, the number showing clinical signs was of the order of 37%, indicating that neuronal loss was not always accompanied by clinical signs.

Of the 31 vaccine lots, representing the three types of virus, that are reported on here, some early batches of type II vaccine exhibited on the whole the greatest intracerebral activity. As shown in Table XI, of 279 monkeys that either survived the observation period or showed some paralysis before death, 11 (3.9%) had

TABLE VI.—Volume and Titres of Successive Lots of Type I Oral Polio Vaccine

Lot No.	Date Prepared	Volume (ml.)	Titre: Log TCD ₅₀ per ml. Undil. TCF
7-1231-110	5/4 57	5,000	7.7
114	9/6 57	2,000	7.5
115	4/9 57	8,000	7.3
120	25/9 57	15,000	7.7
121	7/3 58	20,000	8.2
126	11/7 58	27,000	7.5
140	10/10 58	21,300	7.7
162P	25/3 59	15,000	7.3
Total		113,300	

TABLE VII.—Volume and Titres of Successive Lots of Type II Oral Polio Vaccine

Lot No.	Date Prepared	Volume (ml.)	Titre: Log TCD ₅₀ per ml. Undil. TCF
7-1232-200	15/6 57	2,000	7.5
213	6/9 57	18,000	7.5
216	31/1 58	20,000	7.5
217A	3/3 58	16,000	6.8
222	18/9 58	22,000	7.3
227	10/10 58	13,000	7.3
228	10/10 58	13,000	7.0
240	12/1 59	3,500	7.3
241	12/1 59	5,000	7.6
242	12/1 59	4,000	7.4
243	12/1 59	3,500	7.4
Total		120,000	

TABLE VIII.—Volume and Titres of Successive Lots of Type III Oral Polio Vaccine

Lot No.	Date Prepared	Volume (ml.)	Titre: Log TCD ₅₀ per ml. Undil. TCF
7-1233-310	15/2 57	3,000	7.5
318	26/9 57	10,000	7.7
319	23/11 57	22,000	7.5
334	10/10 58	27,000	6.8
341	4/12 58	4,000	8.0
342	4/12 58	4,500	7.6
344	4/12 58	5,000	7.7
352P	17/12 58	16,000	7.2
360P	17/12 58	16,500	7.1
361P	13/2 59	16,500	7.4
362P	17/2 59	18,500	7.6
Total		143,000	

TABLE IX.—Intracerebral Monkey Virulence of Successive Lots of Type I Oral Polio Vaccine, at Different Dilutions

Lot No.	Titre*	Paralytic Ratio			Polio Histopathological Ratio					
					Cervical Enlargement			Lumbar Enlargement		
		Undil.	10 ⁻¹	10 ⁻²	Undil.	10 ⁻¹	10 ⁻²	Undil.	10 ⁻¹	10 ⁻²
110	7.7	0/3	0/9	0/9	0/5	0/9	0/10	1/4	0/9	0/10
114	7.5	12/10	0/10	0/9	12/10	12/10	0/9	1/10	1/10	0/9
115	7.3	0/10	0/10	1/10	0/10	0/10	12/10	12/10	0/10	12/10
120	7.7	0/10	0/10	0/10	1/10	1/10	0/9	1/10	1/10	0/9
121	8.2	0/9	0/10	0/10	0/9	0/10	0/10	0/9	0/10	0/10
126	7.5	0/10	0/4	0/6	0/10	1/6	0/6	0/10	1/6	0/6
140	7.7	0/10	0/6	0/6	1/9	0/6	0/6	0/10	0/6	0/6
162P†	7.3	0/6	0/4	12/4	0/10	1+12/6	12/6	12/10	2+12/6	2+12/6
Totals		12/68	0/63	1+12/64	2+12/73	3+22/67	22/66	2+32/73	5+12/67	2+22/66
%		1.5	0	3.1	4.1	7.4	3.0	6.8	9.0	6.0
% of Total			1.5			4.8			7.2	

* Log. TCD₅₀ per ml. † Scored histopathologically by method B. All others scored by method A.

weakness or paralysis of a single limb, as against 1.5% for type I vaccines. However, of the 11 affected monkeys, three showed no histopathological lesion whatever, in either the cervical or the lumbar enlargement, and the others showed no greater histological changes than did clinically non-affected animals. Histopathological findings in non-paralysed monkeys also were more frequent for early than for more recent pools of vaccine, and varied in extent among the

different lots. This is illustrated in Table XII, which presents the poliomyelitis histopathological picture of five representative type II vaccine lots.

The lot listed at the bottom of the table is almost completely negative, with but one of the animals showing any neuronal damage in the cervical area, and two in the lumbar. This lot is quite typical of the more recently prepared vaccines, all of which give a greatly reduced neuronal-loss score following intracerebral

TABLE X.—*Intraspinal Monkey Virulence of Successive Lots of Type I Oral Polio Vaccine, at Different Dilutions*

Lot No.	Titre*	Paralytic Ratio			Polio Histopathological Ratio					
					Cervical Enlargement			Lumbar Enlargement		
		Undil.	10 ⁻¹	10 ⁻²	Undil.	10 ⁻¹	10 ⁻²	Undil.	10 ⁻¹	10 ⁻²
110	7.7	6/9	4+17/8	3+17/10	7+12/10	3+17/10	3+37/10	8+17/10	5/10	6+27/10
114	7.5	3/10	4/10	4/10	27/9	6+27/8	4+27/10	4/9	9/10	7/10
115	7.3	3/8	3/8	0/8	3/8	2+17/8	0/8	5/8	4/8	0/8
120	7.7	3/8	4/8	0/7	3+27/8	3+17/8	2/8	6/8	4/7	4/8
121	8.2	2/8	3/6	0/7	1+27/8	2+17/8	17/8	3+27/8	4+17/8	17/8
126	7.5	6/22	3/12	7/17	4+57/22	3+17/11	5+27/18	14+17/21	9/12	10+27/18
140	7.7	11/29	10/15	9/18	6+27/20	3+17/11	5+27/12	16+17/20	9/12	9+17/12
162P†	7.3	2+17/10	3/6	0/6	2+17/10	3/6	0/6	8/10	3+17/6	1+17/6
Totals	..	36+17/104	34+17/73	23+17/83	26+157/95	25+87/70	19+107/80	64+57/94	47+27/73	37+7/80
%	..	35.5	47.9	28.8	43.1	47.1	36.2	73.4	67.1	55.0
% of Total	..	37.0			42.0			65.5		

* Log. TCD₅₀ per ml. † Scored histopathologically by method B. All others scored by method A.

TABLE XI.—*Intracerebral Monkey Virulence of Successive Lots of Type II Oral Polio Vaccine, at Different Dilutions*

Lot No.	Titre*	Paralytic Ratio			Polio Histopathological Ratio					
					Cervical Enlargement			Lumbar Enlargement		
		Undil.	10 ⁻¹	10 ⁻²	Undil.	10 ⁻¹	10 ⁻²	Undil.	10 ⁻¹	10 ⁻²
200	7.5	0/9	0/10	1/10	27/9	1/10	0/10	1+27/9	1/10	0/10
213	7.5	1/8	0/9	0/9	6+27/10	3+47/9	7/10	4+37/10	4+27/9	3+47/10
216	7.5	1/20	1+17/19	1+17/18	3+97/20	5+37/20	3+57/19	7+57/20	6+57/20	4+47/19
217A	6.8	2/19	0/8	0/8	5+57/20	6+27/10	17/10	7+37/20	5+37/10	27/10
222	7.3	0/10	0/5	0/6	27/10	17/6	27/6	27/10	27/6	37/6
227	7.3	0/9	0/6	0/6	9+17/10	6/6	5/6	9/9	6/6	5+17/6
228	7.0	0/10	1/6	0/6	9/9	6/6	9/9	9/9	5/6	4/6
240†	7.3	0/8	0/5	0/4	1/10	0/6	0/6	1/10	0/6	0/5
241†	7.6	0/5	1/5	0/5	17/10	1+17/6	2/6	17/10	2/6	2/6
242†	7.4	0/7	0/5	0/5	17/10	1+17/6	1/6	37/10	1/6	2/6
243†	7.4	0/8	0/6	0/5	1/10	0/6	0/6	1/10	0/6	1/6
Totals	..	4/113	3+17/84	2+17/82	34+237/128	29+127/91	22+87/91	39+197/127	30+127/91	21+147/90
%	..	3.5	4.7	3.6	44.5	45.0	33.0	45.7	46.1	39.0
% of Total	..	3.9			41.3			43.6		

* Log. TCD₅₀ per ml. † Scored histopathologically by method B. All others scored by method A.

TABLE XII.—*Intracerebral Monkey Test: Poliomyelitis Histopathological Score of Representative Lots of Type II Vaccine*

Lot No.	Dilution Inoculated	No. of Monkeys Examined	Polio Histopathological Score*—Number of Monkeys with:													
			Cervical Lesions							Lumbar Lesions						
			0	±	1+	2+	3+	4+	5+	0	±	1+	2+	3+	4+	5+
213†	Undil. 10 ⁻¹ 10 ⁻²	10 9 10	2 2 3	2 4	3 3 3	1 4	2 			3 3 3	2 2 4	1 3 1	4 1 2			
216†	Undil. 10 ⁻¹ 10 ⁻²	10 10 9	5 5 4	3 2 3	2 2 2	1 				5 5 4	1 1 3	4 3 2	1 			
217A†	Undil. 10 ⁻¹ 10 ⁻²	20 10 10	10 2 9	5 2 1	3 5	2 1				10 2 8	3 3 2	4 4	3 1			
227†	Undil. 10 ⁻¹ 10 ⁻²	10 6 6	 1	2 	3 5 3	5 1 1	 1			 	2 2	2 2 2	4 4 2	2 2		
243‡	Undil. 10 ⁻¹ 10 ⁻²	10 6 6	9 6 6	 	 	1 				9 6 5	 	 1	 	1 		
	Totals	142	64	24	34	17	3			63	25	27	22	5		
	%		45.1	16.9	23.9	12.0	2.1			44.4	17.6	19.0	15.5	3.5		

* 0=No cellular loss. ±=Less than 10% loss. 1+=10-20% loss. 2+=21-40% loss. 3+=41-60% loss. 4+=61-80% loss. 5+=80+ loss. † Scored histopathologically by method A. ‡ Scored histopathologically by method B.

inoculation. The discrepancy between results with these and with earlier batches may be due to the fact that some of the individual monkey harvests included in early batches of vaccine were prepared by inoculating culture bottles with diluted seed virus, whereas only undiluted seed has been used during the past year.

Each of the four other lots shown in Table XII was fed to at least 80,000 persons, and one, Lot 213, was fed to almost 200,000. As can be seen, the cervical and lumbar activity of these lots in monkeys seldom reached a 41-60% cell loss (2 to 3.5% of the cases), and in over 60% of the animals findings were either completely negative or minimal (less than 10% loss). Despite the relatively greater neuronal activity of these lots, not a single case of confirmed disease was reported as a result of their ingestion by human beings.

It also is of interest that, as shown in Table XIII, early lots of type II vaccine, though somewhat more active intracerebrally, induced no more paralysis and produced no greater histopathological reactions by the intraspinal route than did type I lots. It may be recalled that for animals showing clinical paralysis or limb weakness following type I inoculations, the rates were 35.5% for the undiluted, 47.9% for the 10^{-1} , and 28.8% for the 10^{-2} groups, or an overall rate of 37%. For type II the corresponding rates were 45%, 41%, and 23%, giving an average rate of 37.6%. Type II vaccines were inoculated intraspinally into a total of 280 monkeys, of which 258 (92.1%) survived the observation period.

Intracerebrally inoculated type III vaccine lots behaved in monkeys very similarly to type I, as indicated in Table XIV. Only 1.2% of the inoculated animals (3 out of the 230 surviving the observation period) showed some weakness of one limb, and all three were histopathologically negative. Histological changes as a whole were minimal, and consisted mostly of inflammatory reactions with little or no neuronal loss.

Intraspinal paralytic rates of type III lots, as shown in Table XV, were 26.7% for the undiluted group and 36% and 31.3% for the 10^{-1} and 10^{-2} dilutions respectively, or an average of 30.7% of the 228 out of 239 surviving animals. The histological damage produced by this route of infection was not very different for type III from that for type I virus.

Discussion

To just what extent can the results obtained in monkeys, particularly with the intraspinal test, predict the safety of attenuated poliovirus for man? This all-important question is difficult, if not impossible, to answer at the present time. Since monkeys now constitute the only available laboratory yardstick, no doubt the association of vaccine strains with the lowest possible degree of neurovirulence in these animals is desirable, so long as it does not entail undue loss of antigenicity. But before a test system can be accepted for routine use, it must be reproducible within a reasonable range of deviation for the formulation of realistic minimum requirements.

TABLE XIII.—*Intraspinal Monkey Virulence of Successive Lots of Type II Oral Polio Vaccine, at Different Dilutions*

Lot No.	Titre*	Paralytic Ratio			Polio Histopathological Ratio					
					Cervical Enlargement			Lumbar Enlargement		
		Undil.	10^{-1}	10^{-2}	Undil.	10^{-1}	10^{-2}	Undil.	10^{-1}	10^{-2}
200	7.5	2/8	1/8	0/8	6+12/10	2+52/10	2+22/9	9/10	6+32/10	3+12/8
213	7.5	4/8	1/8	0/8	6/8	5+22/8	1/8	6/7	7/8	2+12/8
216	7.5	4/8	1/8	0/8	4+12/6	32/8	27/8	5/6	2+12/8	2/8
217A	6.8	6/16	7/16	4/14	9+32/16	12+12/16	4+22/16	13/16	13/16	7/16
222	7.3	3/10	4/6	2/6	5+22/10	1+22/6	1+32/6	9/10	4/6	6/6
227	7.3	5/9	4/6	3/6	5+22/10	5+12/6	3+22/6	7/10	6/6	4+12/6
228	7.0	4/10	4/5	3/5	9+12/10	5+12/6	2+42/6	9+12/10	6/6	4+22/6
240†	7.3	4/10	1/4	1/3	5/10	3/6	1/6	7/10	3/6	3/6
241†	7.6	7/9	4/6	2/6	5+22/10	4+12/5	3+22/6	10/10	5/5	5/6
242†	7.4	7/9	4/5	2/5	8/10	3/6	4/6	9/10	6/6	6/6
243†	7.4	3/10	0/4	0/6	6/9	1/5	1/6	6/9	2/5	2/6
Totals		49/107	31/76	17/75	62+122/109	41+162/82	22+172/83	90+12/108	60+42/82	44+52/82
%		45.0	41.0	23.0	73.4	69.5	47.0	84.3	78.0	59.7
% of Total			37.6			64.2			75.0	

* Log. TCD₅₀ per ml. † Scored histopathologically by method B. All others scored by method A.

TABLE XIV.—*Intracerebral Monkey Virulence of Successive Lots of Type III Oral Polio Vaccine, at Different Dilutions*

Lot No.	Titre*	Paralytic Ratio			Polio Histopathological Ratio†					
					Cervical Enlargement			Lumbar Enlargement		
		Undil.	10^{-1}	10^{-2}	Undil.	10^{-1}	10^{-2}	Undil.	10^{-1}	10^{-2}
310	7.5	0/5	0/4	—	0/5	0/5	—	0/5	0/5	—
318	7.7	0/8	0/9	0/9	0/10	0/10	0/9	0/10	12/10	0/9
319†	7.5	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
334	6.8	0/10	0/6	0/6	0/10	0/6	0/6	0/10	0/6	0/6
341	8.0	12/10	0/6	0/6	1/10	0/6	0/6	1/10	0/6	0/6
342	7.6	0/9	0/6	0/5	0/10	0/6	0/6	0/10	0/6	0/6
344	7.7	0/10	0/6	0/5	0/10	0/6	0/6	0/10	0/6	0/6
352P	7.2	12/10	0/6	0/6	6/9	1/6	1+22/6	5+22/10	1+32/6	1+32/6
360P	7.1	0/8	0/5	0/5	2/10	0/6	0/6	2+12/10	1+12/6	0/6
361P	7.4	0/10	0/5	0/6	2+22/10	0/6	0/6	2/10	0/6	0/6
362P	7.6	12/9	0/4	0/6	22/10	1+12/6	0/5	22/10	0/6	1+12/6
Totals		32/99	0/67	0/64	11+42/104	2+12/73	1+22/66	10+52/105	2+42/73	2+42/67
%		3	0	0	14.4	4.1	4.5	14.3	8.2	8.9
% of Total			1.2			8.6			11.0	

* Log. TCD₅₀ per ml. † Most lesions were scored by method B and consisted mainly of inflammatory reactions with little or no neuronal damage or loss. ‡ Scored histopathologically by method A. All others scored by method B.

Of the two modes of monkey inoculation, the intracerebral test best meets this specification. It is both reproducible and consistent, and should provide a well-founded basis for acceptable criteria of residual neurovirulence. Surely a virus which fails to paralyse and causes only minimal histopathological changes when inoculated into monkeys intracerebrally in amounts equalling or exceeding 10 million TCD₅₀ can be regarded as attenuated several million-fold, in view of the fact that a virulent type I field strain inoculated by the same route did paralyse monkeys with as few as 2 TCD₅₀ (H. R. Cox *et al.*, unpublished observations).

Admittedly, some of the early type II vaccine lots were more active than is desirable when inoculated into monkeys intracerebrally. And though their safety in the field remains unquestioned, steps have been taken to reduce the activity of later lots to a minimum or even virtually to eliminate it. With the more recent batches of type II vaccine this has been accomplished to a great extent.

Compared with the intracerebral test, results with the intraspinal technique have been much less consistent. In fact, considerable variation has been reported from different laboratories (Sabin, 1959; Melnick, 1959). In an attempt to explain these differences, the following factors should be taken into account.

1. *Method of Scoring Lesions.*—In our experience, Methods A and B, previously described, give easily comparable results so far as the intracerebral test is concerned, but not with the intraspinal test. Placement of the inoculum in the anterior horn was not considered crucial when Method A was employed. That method has been used extensively in our laboratory, and gave scores consistently lower than did Method B, which was adopted only recently in order to obtain results comparable to those of other workers.

2. *Size of Needle Used for Inoculation.*—Scores obtained with a smaller (27-gauge) needle were consistently higher than those with a 20-gauge needle, probably because the former expels the virus preparation at greater speed.

3. *Number of Inoculations.*—More extensive lesions were obtained with the "multiple jab" method, as employed by Melnick, than with the single-inoculation technique of Sabin, a fact already stressed by Melnick (1959). Obviously, with the former method the chances of "hitting" the

anterior horn, often repeatedly, are greater than with the latter.

4. *Placement of Inoculum Within Spinal Cord.*—In his description of the intraspinal test, Sabin (1959) emphasized the necessity of inoculating the virus within the anterior horn, in direct contact with the large motor-nerve cells, as would be indicated by a needle track extending from the posterior or posterior-lateral aspect of the cord through the anterior horn to the anterior lateral cord surface. However, histological determination of whether the inoculum was being placed in the anterior horn or in the

FIG. 1.—Intraspinal inoculation of cynomolgus monkeys. Left: Needle track in white matter—neurons intact—no inflammation. Right: Needle track in grey matter—neurons destroyed—marked inflammation.

posterior position of the track often proved difficult, and a difference in position of a few microns would result in considerable difference in the scoring of the lesion.

It may be pertinent at this point to raise the question of the significance of a track outside the anterior horn. Fig. 1 shows, side by side, sections from the lumbar cords of two monkeys inoculated with the same dilution of type II vaccine. In the section to the right the needle track is properly placed in the grey matter, the adjacent area shows marked inflammation, and in both horns almost all neurones are destroyed. In the one to the left the track is clearly visible in the white matter, and in the adjacent anterior horns all neurones have been spared. Sabin (1959) proposed, in his discussion of the intraspinal test, that in the final evaluation of results those animals yielding cord sections with the needle track in the white matter be disregarded. But instead, shouldn't a finding such as that depicted in the photograph raise the following question? How virulent or invasive can a poliovirus be if it cannot bridge a gap

TABLE XV.—Intraspinal Monkey Virulence of Successive Lots of Type III Oral Polio Vaccine, at Different Dilutions

Lot No.	Titre*	Paralytic Ratio			Polio Histopathological Ratio					
					Cervical Enlargement			Lumbar Enlargement		
		Undil.	10 ⁻¹	10 ⁻²	Undil.	10 ⁻¹	10 ⁻²	Undil.	10 ⁻¹	10 ⁻²
310†	7.5	0/5	22/5	3/5	1/5	1+37/5	1/5	1+12/5	5/5	2+12/5
318†	7.7	3/8	3/8	0/8		3+12/8	1/8	7/8	5/8	5/8
319†	7.5	1/8	1/8	1/8	22/8	37/8	1/8	4+12/8	1+12/8	2+12/8
334	6.8	4/9	3/5	1/6	0/10	2/6	0/6	7/10	5/6	2/6
341	8.0	1/10	2/6	3/6	1+22/10	22/6	12/6	5+12/10	4/6	3+22/6
342	7.6	5/10	2+12/5	4/6	3+32/9	5+12/6	4+22/6	9+12/10	6/6	5+12/6
344	7.7	1/10	0/5	0/5	1/10	4/6	0/6	5+22/10	5+12/6	1+12/6
352P	7.2	0/9	4/6	4/6	1/10	3+12/6	2+12/6	8+12/10	6/6	6/6
360P	7.1	0/10	0/4	0/6	2/9	12/6	1/6	8/9	6/6	4/6
361P	7.4	4/9	2/6	3/6	5+22/9	3/6	2/6	8/9	4/6	5/6
362P	7.6	7/9	2+12/6	1+12/5	4/10	0/6	1/6	10/10	4/6	4/6
Totals	..	26/97	19+42/64	20+12/67	21+92/98	21+122/69	13+42/69	72+72/99	51+22/69	39+62/69
%	..	26.7	36.0	31.3	30.5	47.8	24.6	79.7	76.8	65.2
% of Total	..		30.7			33.9			74.6	

* Log. TCD₅₀ per ml. † Scored histopathologically by method A. All others scored by method B.

of a few millimetres, when virulent poliovirus placed anywhere in the central nervous system, and even extraneurally, is well known to be capable of crossing more formidable barriers in seeking out and destroying susceptible neurones.

5. Histological Interpretation of Circumscribed Lesions as Specifically Due to Virus Action.—Typical non-specific traumatic lesions are shown in Fig. 2. The lesion on the left is undoubtedly traumatic, as indicated by the extensive

FIG. 2.—Intraspinal inoculation of cynomolgus monkeys. Left: Traumatic lesion with formation of a small area of softening and intact neurones in anterior horns. Right: Extensive area of softening in grey matter with secondary inflammation and possible specific viral effect.

formation of scavenger cells and the secondary inflammatory reaction at the periphery of the softening. On the right, a similar lesion can be seen in the anterior horn which might be interpreted either as purely traumatic or as a mixture of trauma and specific viral effect.

In other instances, non-specific reactions to foreign material may present similar difficulty in interpretation. The occurrence of such reactions following intraspinal inoculation is well established. In Fig. 3, for example, the presence of large multinucleated cells indicates that the

FIG. 3.—Intraspinal inoculation of cynomolgus monkeys. Section of lumbar enlargement showing large multinucleated cells in the grey matter, indicating inflammatory reaction due to foreign bodies and not to poliovirus.

inflammatory reaction was caused by foreign bodies and not by poliovirus. In other cases, however, the interpretation might remain in doubt.

6. Possibility of Introduction with Inoculum of a Bacterial Agent of Low Virulence.—The formation of small abscesses with an accumulation of polymorphonuclear cells was seen occasionally, as were perivascular infiltrations, which included large numbers of leucocytes. These haematogenous elements are well known to appear only in the very first phase of the poliomyelitis process and to disappear

almost completely by the third week (the time at which the monkeys were sacrificed).

At this point it may be of interest to report results of recent experiments intended to evaluate the reproducibility of the intraspinal test when using an inoculation technique as uniform as possible. This was done in two ways: by repeated testing of the same vaccine lot, or by simultaneous testing of vaccine lots prepared at the same time from the same seed lot. Results of these tests are summarized in Tables XVI and XVII.

In Table XVI, three parallel lots of type III vaccine, inoculated by means of a 20-gauge needle, gave paralytic ratios of 6/22 monkeys for the first, 12/21 for the second, and 1/20 for the third. This variation is notable even if, as proposed

by Sabin, results excluded those animals which on histological examination showed no needle track or showed tracks in the white matter. The respective rates for the three lots then become 6/12, 12/21, and 1/17, or percentages of 37.5, 54.5, and 6, a noteworthy variation. The discrepancy may reflect the inherent irreproducibility of the test despite uniform technique, or may indicate that neurovirulence of the virus in different production lots can fluctuate appreciably despite uniform production conditions. Should the latter be the case, it becomes imperative to test several serial lots of any new vaccine before its intraspinal performance can be properly evaluated.

However, the importance of taking into account the first possibility mentioned above—irreproducibility of the test—is suggested by the experiment described in Table XVII. Here the same lot of type III virus was tested intraspinally, twice with a 20-gauge and once with a 27-gauge needle. Excluding the animals in which the inoculum was not placed in the grey matter, paralytic ratios in the two 20-gauge-needle tests were 1/17 (6%) and 8/14 (57%), respectively, almost a tenfold difference. The 27-gauge-needle test confirmed the greater reaction elicited by this technique, almost 100% of the animals showing some degree of limb weakness or paralysis.

While the intraspinal technique undoubtedly can be a valuable experimental tool for the selection of poliovirus variants completely devoid of their affinity for neurones, the findings reported above give reason for believing that it should not be a requirement for oral poliomyelitis vaccine. First, the true relation of the results of the test to the vaccine's safety for man is obscure. Second, how could the safety of a vaccine be measured with any degree of uniformity by a test subject to such wide variations which, in all probability, would be carried out by many hands in several laboratories?

This laboratory recently has segregated virus strains of notably less intraspinal activity, derived from isolates other than those described in this report. However, in view of the questionable significance of the intraspinal test, it would be unwise to consider at this time the substitution of untried candidates for strains already proved safe in the field. Furthermore, in the final assessment of vaccine strains, factors other than monkey neurovirulence must also be considered. Antigenicity, for one, is as important as safety, and the strains reported on here have been shown to elicit very satisfactory immune responses, while viruses with less intraspinal activity might prove immunogenically inferior. In addition, there is increasing evidence that the Lederle strains can be administered simultaneously in a trivalent preparation, with immunogenic results equivalent to those following monovalent feedings (Cox *et al.*, 1959).

And isn't it in man himself that the safety of oral poliomyelitis vaccine is ultimately established, however attenuated a poliovirus may be found to be in the laboratory? Data accumulated so far with the Lederle strains of virus are beginning to answer this question. Complete results of all clinical trials carried out with these strains are not yet available, but those now at hand are most reassuring.

Of approximately 500,000 persons fed type I virus (Table XVIII), antibody surveys in four trials comprising 65,429 individuals, or about 12% of the total fed, revealed that 13,959 (21.3%) of them lacked type I antibodies. The rate of homotypic negatives varied widely among areas, ranging from 10% of the study group in Cuba to 44% in Minnesota. If we assume the applicability of the lowest rate, 10% to all persons fed, it can be estimated that of the total number of 500,000, 50,000 were negative for type I at the time

TABLE XVI.—*Variation of Intraspinal Monkey Virulence on Simultaneous Testing of Consecutive Lots of Type III Oral Polio Vaccine*

Lot No.	Needle Gauge	Dilution	Paralytic Ratio	Polio Histopathological Ratio with Needle Track					
				Absent		In White Matter		In Grey Matter	
				C.	L.	C.	L.	C.	L.
341	20	Undil. 10 ⁻¹ 10 ⁻²	1/10 2/6 3/6	0/3 0/2 0/1	0/3 — 0/1	— — —	— — —	1+22/7 22/4 12/5	5+12/7 4/4 3+22/5
342	20	Undil. 10 ⁻¹ 10 ⁻²	5/10 2+12/5 4/6	— — —	— — —	— — —	— — —	3+32/9 5+12/6 4+22/6	9+12/10 6/6 5+12/6
344	20	Undil. 10 ⁻¹ 10 ⁻²	1/10 0/5 0/5	0/1 — 0/2	0/1 — 0/2	0/1 — 0/1	0/1 — 0/1	1/8 4/6 0/3	5+22/8 5+12/6 1+12/3

TABLE XVII.—*Variation of Intraspinal Monkey Virulence on Repeated Testing of Same Lot of Type III Oral Polio Vaccine (Lot 7-1233-344)*

Test No.	Needle Gauge	Dilution	Paralytic Ratio	Polio Histopathological Ratio with Needle Track					
				Absent		In White Matter		In Grey Matter	
				C.	L.	C.	L.	C.	L.
1	20	Undil. 10 ⁻¹ 10 ⁻²	1/10 0/5 0/5	0/1 — 0/2	0/1 — 0/2	0/1 — 0/1	0/1 — 0/1	1/8 4/6 0/3	5+22/8 5+12/6 1+12/3
2	20	Undil. 10 ⁻¹ 10 ⁻²	4/10 3/6 1/4	0/2 0/1 0/4	0/2 0/1 0/4	0/1 — —	0/1 — —	4/7 4/5 1/2	7/7 5/5 2/2
3	27	Undil. 10 ⁻¹ 10 ⁻²	9/10 5+12/6 1+22/5	— 0/1 —	— 0/1 —	1/1 0/1 0/2	1/1 0/1 2/2	7/9 0/4 1/4	7/9 4/4 3/4

TABLE XVIII.—*Field Experience with Successive Lots of Type I Oral Polio Vaccine*

Lot No.	Log. TCD ₅₀ Fed	Country or State	No. of Persons		Estimated Negatives			
			Fed	Tested	Homotypic		Triple	
					No.	%	No.	%
110	5.0	Minnesota: 2nd trial ..	551	551	241	44.0	113	20.5
110	5.0	Uruguay	45,000					
115	5.7	Andes, Colombia	7,378	452	2,088	28.3	952	12.9
115	5.7	Costa Rica	28,000					
115	5.7	Uruguay	9,000					
120	5.1	Cuba	2,000	274	200	10.0	20	1.0
120	5.1	Uruguay	130,000					
121	5.5	Medellin, Colombia ..	133,000					
121	5.5	Haiti	5,000					
121	5.5	Nicaragua	55,500	505	11,430	20.6	1,665	3.0
121	5.5	Uruguay	60,000					
Total fed			475,429					
Total surveyed			65,429	1,782	13,959	21.3	2,750	4.2

of ingestion of the vaccine. In addition, there were in the four completed trials, an estimated 2,750 triply negative individuals. These rates also differed substantially, ranging from 1% in Cuba, and 3% in Nicaragua, to 13% in Andes, Colombia, and 20.5% in the Minneapolis study.

Though the rate of triple negatives before ingestion remains the same for type II vaccine, the rate of homotypic negatives in the four trials was lower: 7,208 persons, or 11% of the total (Table XIX). Again, rates differed greatly according to area, ranging from 5.5% in Cuba to 33% in Andes, Colombia.

Table XX summarizes the results obtained with type III virus. The number of homotypic negatives was estimated at 14,072, representing a rate very close to that for type I.

The numbers of homotypic and triple negatives in these studies appear to be sufficiently great to have caused some difficulties had the viruses used not been attenuated. However, as will be reported by others, not a single proved case of paralysis attributable to the vaccine was reported from any of the trial areas. This was equally true for type II virus despite its somewhat greater activity in monkeys. It should also be borne in mind that the numbers cited apply only to persons vaccinated, without taking into account the probably even greater numbers of the satellite infections that undoubtedly have occurred as a result of contact exposure.

Summary and Conclusions

Brief histories of the three type strains of Lederle attenuated polioviruses have been presented, and

production and testing data pertaining to 31 lots of experimental oral vaccine have been described. In all, 113 litres of type I vaccine, 120 of type II, and 143 of type III were prepared and completely tested, yielding quantities sufficient for 4½, 2½, and 4½ million human immunizations, respectively.

Only an occasional bacterial contaminant, and no C.N.S., herpes, simian, measles, or B virus was encountered. The presence of tubercle bacilli was also excluded from all vaccine lots, both by guinea-pig inoculation and in culture media.

Intracerebral monkey inoculations of these vaccines produced overall paralytic rates of 1.5% for type I, 3.9% for type II, and 1.2% for type III. Histopathological changes following inoculation by this route were virtually absent with type I and type III vaccine lots. Though early batches of type II were associated with some neuronal loss, more recent lots have produced almost none.

Despite the somewhat greater activity of some type II vaccines when inoculated intracerebrally, in monkeys inoculated intraspinally activity of lots of all three types was comparable, producing similar overall paralytic rates. Paralysis induced by this route of inoculation was usually non-progressive and limited to one or both lower limbs, and only an occasional animal died within the 21-day observation period. Histopathological lesions were observed in most intraspinally inoculated animals, whether or not they were paralysed.

Each of the three Lederle strains of virus has been fed to approximately 500,000 persons in several trials in the U.S.A. and Central and South America. In

TABLE XIX.—*Field Experience with Successive Lots of Type II Oral Polio Vaccine*

Lot No.	Log. TCD ₅₀ Fed	Country or State	No. of Persons		Estimated Negatives			
					Homotypic		Triple	
			Fed	Tested	No.	%*	No.	%*
213	5.2	Andes, Colombia ..	7,122	452	2,357 141	33.1 25.5	952 113	12.9 20.5
213	5.2	Minnesota: 2nd trial ..	551	551				
213	5.2	Uruguay	160,000					
216	5.5	Medellin, Colombia ..	48,000	505	4,600	8.3	1,665	3.0
216	5.5	Nicaragua	55,500					
216	5.5	Uruguay	90,000					
217A	5.4	Medellin, Colombia ..	85,000					
227	5.7	Cuba	2,000	274	110	5.5	20	1.0
227	5.7	Costa Rica	75,000					
227	5.7	Haiti	5,000					
Total fed			528,173					
Total surveyed			65,173	1,782	7,208	11.0	2,750	4.2

* Percentages are based on total number of persons starting course of feedings.

TABLE XX.—*Field Experience with Successive Lots of Type III Oral Polio Vaccine*

Lot No.	Log. TCD ₅₀ Fed	Country or State	No. of Persons		Estimated Negatives			
					Homotypic		Triple	
			Fed	Tested	No.	%*	No.	%*
318	5.3	Andes, Colombia ..	6,977	457	2,728 356	39.1 17.8	952 20	12.9 1.0
318	5.3	Cuba	2,000	274				
318	5.3	Medellin, Colombia ..	50,000					
318	5.3	Minnesota; 2nd trial ..	551	551	10,670	57.7 19.4	113 1,665	20.5 3.0
318	5.3	Nicaragua	55,500	505				
318	5.3	Uruguay	58,000					
319	5.5	Medellin, Colombia ..	83,000					
319	5.5	Haiti	5,000					
319	5.5	Uruguay	192,000					
Total fed			453,028					
Total surveyed			65,028	1,787	14,072	21.6	2,750	4.2

* Percentages are based on total number of persons starting course of feedings.

completed trials comprising 65,429 individuals, or 12% of the total fed, estimated homotypic negatives amounted to 13,959 or 21.3% for type I, 7,208 or 11% for type II, and 14,072 or 22% for type III. In addition there were, among the 65,429 persons surveyed serologically, an estimated 2,750 triple negatives. In not a single instance was a proved case of paralysis or disease attributed to the vaccine.

The intraspinal test has been discussed with regard to its reproducibility and its significance in relation to safety of the vaccine virus for man. The more consistent and more readily carried out intracerebral monkey inoculation has been proposed as the basis for criteria of residual monkey virulence to be used for release of oral vaccine.

Efforts to improve Lederle vaccine strains in every possible way are continuing. Meanwhile the utility of the present strains, in view of the obvious need for a better solution to the problem of immunization against paralytic poliomyelitis, has been demonstrated by the mounting evidence of their effectiveness and safety and the absence of significant undesirable reactions.

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The American Department of Health Education and Welfare has recently issued a bibliography of selected references in the English language during 1952–8: *Maternal Disorders Related to Fetal Stress, Perinatal Death, and Congenital Defects*. It is limited in scope to the following subjects: effects of maternal infectious diseases, diabetes, thyroid dysfunction, myasthenia gravis, lupus erythematosus, sickle-cell disease, thrombocytopenic purpura, leukaemia, drug addiction, and drug administration. A number of important statistical studies of perinatal morbidity and mortality are also included. A limited number of copies of the bibliography are available from the Library, National Institutes of Health, Bethesda, 14, Maryland, U.S.A.

Q FEVER IN THE R.A.F. IN GREAT BRITAIN IN 1958

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The epidemiology of Q fever has been fully reviewed by Marmion and Stoker (1958). The present report deals with two localized outbreaks, both of which illustrate an interesting facet of this disease, and with a general investigation into the prevalence of Q fever in Royal Air Force personnel in this country. Acute respiratory disease in the R.A.F. has been investigated in recent years in collaboration with the Central Public Health Laboratory, Colindale (Andrews *et al.*, 1956; McDonald *et al.*, 1958; Holland *et al.*, 1959). No case of Q fever has been detected in the recruit units, in which there is a very high prevalence of acute respiratory disease, and few in operational units though many pairs of sera have been tested.

The outbreaks of Q fever occurred at a large Coastal Command station in Cornwall and in a small officers' training unit in the Isle of Man in March–April, 1958. No relation between these two outbreaks could be found. Investigation of the prevalence of Q fever in other stations was limited to 20 operational stations, as the recruit and trade training centres had revealed no cases by the routine investigations of respiratory disease in the R.A.F.

Laboratory Methods

The complement-fixation tests were carried out in the cups of plastic haemagglutination plates (World Health Organization, 1953). The antigen used was prepared by the Standards Laboratory, Colindale, from the Nine Mile strain of *Rickettsia burnetii*.

Serum dilutions, complement ($2\frac{1}{2}$ H.D.₅₀), and antigen were mixed in 0.1-ml. volumes and allowed to fix overnight at 4° C. Next morning the plates were put in an incubator at 37° C. for half an hour, 0.1 ml. of a 2% suspension of optimally sensitized sheep red blood cells was added, and after a further 30 minutes at 37° C. the plates were transferred to a refrigerator at 4° C. to allow unlysed cells to settle. The titre recorded was the highest serum dilution showing 75% or more of the red cells unlysed.

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